IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Ammermann et al.
Serial No.	10/509,635
Filed:	April 07, 2003
Group Art Unit:	1616
Examiner:	Danielle Sullivan

For: Method for increasing the resistance of plants to the phytotoxicity of agrochemicals

DECLARATION

1. I, Lutz Brahm, Dr. agr., citizen of the Federal Republic of Germany and residing at: Am Hang 16, 67551 Worms, Germany, hereby declare as follows:

   I am a fully trained Agronomist having studied Agriculture at the Justus-Liebig-University of Giessen, Germany, from 1987 to 1993. I received a Diploma Degree in 1993 by the Justus-Liebig-University of Giessen, Germany. In 1997, I received the doctorate degree (Ph.D.) by the Justus-Liebig-University of Giessen, Germany.

   I joined BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany, in 2006. Since then, I have been working in the field of crop protection. I have read and fully understood US application Ser. No. 10/509,635 and I am familiar with the subject-matter disclosed and claimed therein;

2. I have read and fully understood the Office Action of April 01, 2010 and the references cited therein by the Examiner;

3. The following observations are made by me.

4. Supplementary Experimental Data

In order to provide further support for the claimed method, following additional test data are presented.

Soybeans were grown in 2010 in the greenhouse at the agricultural center at Limburgerhof, Germany. The variety DKC25-52 RR2 was planted in pots. The trial was setup with 10 replications with one pot 5 plants each per replication.

The active ingredients were used as formulations. The formulations were used in the product rates given below and in Table 1 and Table 2. The products were applied in a total spray volume of 375 l/ha. Products were diluted in water. The spray solution was applied in a spray cabinet using a spray boom with flat fan nozzles.

Glyphosate was applied twice as Roundup® (360 g active per liter) at soybean growth stage 12 (BBCH) and 13 (BBCH) with a product rate of 3.125 l/ha at each application (1,125 g active per ha). The term "BBCH growth stage" refers to the extended BBCH-scale which is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species in which the entire developmental cycle of the plants is subdivided into clearly recognizable and distinguishable longer-lasting developmental phases. The abbreviation BBCH derives from the Federal Biological Research Centre for Agriculture and Forestry (Germany), the Bundessortenamt (Germany) and the chemical industry. Pyraclostrobin was applied once at growth stage 13 (BBCH) as HEADLINE® (250 g active per liter) with a product rate of 0.1 l/ha (25 g active per ha). At growth stage 13 (BBCH) Roundup® and HEADLINE® were tank mixed.

Relative chlorophyll content (Minolta SPAD 502) was measured 5 days after the second treatment (table 1). Total shoot biomass was assessed (table 1) by harvesting all plants of a pot 17 days after last treatment and is expressed as g per pot. Both fresh and dry weight of total shoot biomass per pot was evaluated. After measuring fresh weight, the samples were dried in a drying cabinet at 65°C until no more change in weight was observed. The efficacy was calculated as % increase of biomass in the treatments compared to the untreated control:

$$E = (a/b-1) \cdot 100$$

a corresponds to the biomass of the treated plants in g/pot and

b corresponds to the biomass of the untreated (control) plants in g/pot

An efficacy of 0 means the yield level of the treated plants corresponds to that of the untreated control plants; an efficacy of 100 means the treated plants showed a biomass increase of 100%. A negative efficacy value means a decrease in biomass compared to the control.

Table 1: Effect of glyphosate and pyraclostrobin application at growth stage 12 and 13 (BBCH) on fresh total shoot biomass of potted soybeans

Treatment	AI rate [g/ha]	Shoot fresh weight [g/pot]	Efficacy [%]
Control	-	11.363	
Pyraclostrobin	25	11.03	-2.9
Glyphosate	2250	8.282	-27.1
Glyphosate + Pyraclostrobin	2250 25	11.565	1.8

Table 2: Effect of glyphosate and pyraclostrobin application at growth stage 12 and 13 (BBCH) on dry total shoot biomass of potted soybeans

Treatment	AI rate [g/ha]	Shoot dry weight [g/pot]	Efficacy [%]
Control	-	1.521	
Pyraclostrobin	25	1.541	1.3
Glyphosate	2250	1.057	-31.4
Glyphosate + Pyraclostrobin	2250 25	1.641	7.9

Despite the phytotoxic impact of glyphosate at the applied dose rates on crop growth, proving the growth inhibiting effect of glyphosate, the effect of the mixture of glyphosate and pyraclostrobin is quite remarkable. There is no negative effect observed in this mixture at the given ratio of the products as can be expected from the huge growth inhibiting impact of glyphosate on soybean growth. The negative impact of glyphosate on biomass production is

totally compensated leading to even a biomass increase by the mixture compared to untreated.

Similarly, corn variety DKC 61-72RR2 was planted with 5 plants per pot and grown in the greenhouse at the BASF agricultural center Limburgerhof. Again, each treatment consisted of 10 replications of one pot.

The active ingredients were used as formulations. The formulations were used in the product rates given below and in table 3 and table 4. The products were applied in a total spray volume of 375 l/ha. Products were diluted in water. The spray solution was applied in a spray cabinet using a spray boom with flat fan nozzles.

Glyphosate was applied twice as Roundup® (360 g active per liter) at corn growth stage 12 (BBCH) and 13 (BBCH) with a product rate of 3.125 l/ha at each application (1125 g active per ha). Pyraclostrobin was applied once at growth stage 13 (BBCH) as HEADLINE® (250 g active per liter) with a product rate of 0.1 l/ha (25 g active per ha). At growth stage 13 (BBCH) Roundup® and HEADLINE® were tank mixed.

Plant height was measured 7 and 17 days after the last treatment (DALT, table 3). At 7 DALT plant height was measured as the length of the shoot from soil surface to the knot of the third leaf. At 17 DALT plant height was measured as shoot length from soil surface to the knot of the fourth leaf. Total shoot biomass was assessed (table 4) by harvesting all plants of a pot 17 days after last treatment and is expressed as g per pot. Both fresh and dry weight of total shoot biomass per pot was evaluated. After measuring fresh weight, the samples were dried in a drying cabinet at 65°C until no more change in weight was observed. The efficacy was calculated as % increase of plant height and biomass in the treatments, respectively, compared to the untreated control:

$$E = (a/b-1) \cdot 100$$

a corresponds to the plant height (cm) or biomass (g/pot) of the treated plants and

b corresponds to the plant height (cm) or biomass (g/pot) of the untreated (control) plants

An efficacy of 0 means the yield level of the treated plants corresponds to that of the untreated control plants; an efficacy of 100 means the treated plants showed an increase in plant height or biomass of 100%. A negative efficacy value means a decrease in plant height or biomass compared to the control.

Table 3: Effect of glyphosate and pyraclostrobin application at growth stage 12 and 13 (BBCH) on plant height

Treatment	AI rate [g/ha]	Plant Height 7 DALT (cm)	Efficacy 7 DALT [%]	Plant Height 17 DALT (cm)	Efficacy 17 DALT [%]
Control	-	13.69		19.93	
Pyraclostrobin	25	13.80	0.8	21.21	6.40
Glyphosate	2250	11.26	-17.75	17.52	-12.09
Glyphosate + Pyraclostrobin	2250 25	13.92	1.8	25.34	27.14

Table 4: Effect of glyphosate and pyraclostrobin application at growth stage 12 and 13 (BBCH) on fresh and dry total shoot biomass of potted corn plants

Treatment	AI rate [g/ha]	Shoot fresh biomass [g/pot]	Fresh biomass: efficacy [%]	Shoot dry biomass [g/pot]	Dry biomass: efficacy [%]
Control	-	51.78		3.05	
Pyraclostrobin	25	60.25	16.4	3.86	26.60
Glyphosate	2250	47.15	-8.9	2.99	-1.97
Glyphosate + Pyraclostrobin	2250 25	56.84	9.8	3.56	16.72

Despite the phytotoxic impact of glyphosate at the applied dose rates on crop growth, proving the growth inhibiting effect of glyphosate, the effect of the mixture of glyphosate and pyraclostrobin is quite remarkable. There is no negative effect observed in this mixture at the given ratio of the products as can be expected from the huge growth inhibiting impact of glyphosate on soybean growth. The negative impact of glyphosate on plant height and biomass production is totally compensated leading to even an increase in plant height and biomass by the mixture compared to untreated.

As can be seen from the examples above and the examples in the patent publication US 2005/0164882 this effect is observed in different crops (soybeans, maize, rice). It can be concluded that the safening effect of pyraclostrobin is a general response across crop species. However, unexpectedly, that effect could not be observed in the weed species in the examples given in US 2005/0164882 (*Echinochloa crus-galli*, *Chenopodium album*, *Pharbitis purpurea*).

The examples given here show clearly that pyraclostrobin does not have a negative effect on the crop when applied alone. It is not to be expected that pyraclostrobin in the examples given in US 2005/0164882 has an effect that is different to what is observed here.

The examples given in US 2005/0164882 and here demonstrate that pyraclostrobin compensates the phytotoxic impact of herbicides with a different mode of action in plants. It can therefore be concluded that this effect of pyraclostrobin is not specifically effective in mixture with specific herbicides of a certain mode of action in plants but can be anticipated to be a general safening effect.

5. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1101 of Title 18 of the US-code and that such willful false statements may jeopardize the validity of the above-identified patent issued thereon.

Ludwigshafen, July 09, 2010 .....

(Dr. Lutz Brahm)